

The Hamster Flank Organ Model: Is it Relevant to Man?

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The critical role that androgens play in the etiology of acne has led to a search for topically active antiandrogens and the frequent use of the flank organ of the golden Syrian hamster as an animal model. 17- α -propyltestosterone (17-PT) has been identified as having potent antiandrogenic activity in the hamster model, and this report describes its clinical evaluation. Two double-blind placebo controlled studies comparing 4% 17-PT in 80% alcohol versus vehicle alone were conducted. One study examined 17-PT sebosuppressive activity in 20 subjects. The second study examined its efficacy in 44 subjects having mild to moderate acne. A third study

measured in vitro percutaneous absorption of 17-PT through hamster flank and monkey skin, and human face skin in-vivo, using radioactive drug. 17-PT was found to be ineffective in reducing either the sebum excretion rate or the number of inflammatory acne lesions. Failure of 17-PT to show clinical activity was not a result of poor percutaneous absorption. Total absorption in man was 7.7% of the dose and only 1.0% in the hamster. The sebaceous gland of hamster flank organ is apparently more sensitive to antiandrogens than the human sebaceous gland. *J Invest Dermatol* 93:475-479, 1989

For many years now the critical role of androgens in the etiology of acne has been recognized [1] and this has led to a search for pharmacologic agents that selectively block the action of androgens on the sebaceous gland. Inasmuch as the systemic use of antiandrogens is likely to be accompanied by significant adverse reactions secondary to effects on other androgen sensitive structures, the search for efficacious topical agents has become the more rational approach.

Following a systematic screening of androstane and androstene analogues of testosterone, Ferrari et al [2] reported on the discovery of a compound, 17- α -propyltestosterone (17-PT), which effectively suppressed androgen-induced enlargement of the sebaceous gland in two animal models, the flank organ of the Syrian hamster and the supracaudal gland of the guinea pig. Topical application of this compound to mature male hamsters induced a reversible regression of the flank organ in a dose-dependent fashion with no evidence of systemic activity. The effect was seen with doses ranging from 10-250 μ g/day, incorporated in 5 μ l ethanol and directly applied to the skin overlying the flank organ. The mechanism of action of this drug is thought to be direct blockade of dihydrotestosterone binding to tissue receptors [3].

Although 17-PT was later clinically evaluated in the treatment of acne, it was found to be without efficacy in man, and the results were never published. Recently, preliminary publication [4,5] of data on a second drug (Ro 15-0778), which was found to suppress the sebaceous gland in animal models but was without effect in man, has been noted. This has prompted us to report the data originally obtained on 17-PT and to question the continued use of the hamster flank organ model for the study of the human sebaceous gland.

The data presented here are derived from three separate studies. The percutaneous absorption of 17-PT has been evaluated in a series of in vitro and in vivo studies, and clinical evaluations of both sebum suppression and efficacy in acne have been conducted in human volunteers.

MATERIALS AND METHODS

17-PT (17- α -propylandroster-4-en-17 β -ol-3-one, WIN 17,665) was supplied by Sterling-Winthrop Research Institute (Rensselaer, NY) (Fig 1).

The formulation used in all studies involving topical application of the drug was 4% (w/v) 17-PT in a vehicle consisting of 80/20 ethanol/water. For the acne and sebum suppression studies the formulation was supplied as saturated fiber pads, packaged 42 per jar. For the percutaneous absorption and bioavailability studies, carbon-14 labeled 17-PT, specific activity of 3.7 μ Ci/mole, was incorporated into the standard stock formulation in tracer amounts. The one exception involved subcutaneous injection in the monkey, where only radioactive drug was used to take advantage of a higher specific activity. In this case a 1.2% solution of drug in 95% ethanol was used.

Approval to conduct the in vivo studies was obtained from the appropriate Human Subjects Investigational Review Boards. Written informed consent was obtained from all subjects including parental consent where necessary.

Percutaneous Absorption Studies In vitro percutaneous absorption studies were conducted using a previously published technique [6]. Essentially, the method consists of mounting full-thickness skin on specially constructed diffusion chambers (Crown Glass

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Abbreviations:

N/C: nodules/cysts

Pap: papule

Pust: pustule

Ro 15-0778: (E)-1,2,3,4,-tetrahydro-1,1,4,4,-tetramethyl-6-(1-methyl-2-phenylethenyl)naphthalene

SER: sebum excretion rate

17-PT: 17- α -propyltestosterone

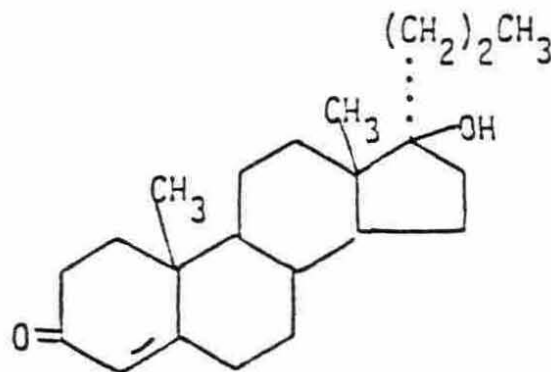


Figure 1. Chemical structure of 17- α -propyltestosterone.

Co., Somerville, NJ). The dermis is bathed by isotonic saline (pH 7.4), maintained at 37°C, and stirred magnetically. The epidermis is open to the ambient laboratory environment. Serial chamber samples were collected and analyzed spectroradiometrically.

Hamster: Six mature male Syrian hamsters (Charles River Laboratories) were killed by Nembutal injection and the hair on the back closely shaved with electric clippers. Duplicate full-thickness specimens from both flank organ-containing skin and non-flank organ skin were taken from each animal and mounted on diffusion cells in which 1.0 cm² of skin surface was available for study. Following a 1-h equilibration period, 10 μ l of radioactive test solution was applied to the epidermal surface, and absorption was assessed by removing the dermal bathing solution in toto at regular intervals and analyzing for radioactive content.

Monkey: Back skin from three monkeys (*Macaca nemestrina*) was obtained through the University of Washington Regional Primate Center Tissue Program. Skin was prepared, mounted onto diffusion chambers, and percutaneous absorption of 17-PT was performed as described above.

Subcutaneous injection of 17-PT was also performed in the rhesus monkey in order to determine fractional urinary excretion, a factor needed for the calculation of total absorption following topical application in man (see below). Three adult rhesus monkeys (*Macaca mulatta*) were tranquilized with Ketamine; a portion of their backs was shaved with electric clippers; and the animals were placed in metabolism chairs (Plas-Labs, Lansing, MI). Radioactive 17-PT, approximately 10 μ Ci in 0.075 ml ethanol, was injected into the subcutaneous space of the back using a microsyringe. Total urinary output was collected for 5 d and analyzed for radioactive content to determine the fractional dose excreted in the urine.

Human: Human abdominal skin was obtained at autopsy from three adult donors, mounted on diffusion chambers, and percutaneous absorption of 17-PT was performed as previously described.

The *in vivo* percutaneous absorption of 17-PT was evaluated in five healthy young adult male subjects. The subjects were prohibited from using topical medications for 1 week prior to the start of the study. None of the subjects had used topical or systemic retinoids.

On the morning of the first study day, 15 min prior to dosing, control urine specimens were collected from each subject and a mild soap (Alpha-Keri) and water wash of the face completed. Then 0.1 ml of radioactive test solution was applied by micropipette to 50 cm² face skin, equally divided between cheek and forehead, and evenly spread with a glass rod. The subjects were allowed to go about their normal daily activities but were instructed not to rub or wash their faces and to avoid activities that would stimulate sweating. Radioactive drug remained on the face for 10 h, then was washed off with soap and water.

Total urinary output was collected for 5 d at 2.5-h intervals for the first 10 h and at approximately 12-h intervals thereafter. Five milliliters of each urine specimen was gelled with 10 ml scintillation fluid (Aquasol-II) and counted in a liquid scintillation spectrometer. Quenching was corrected by the external standard method. Particularly dark urine specimens were first diluted with distilled water prior to sampling.

Ten-milliliter blood samples were obtained at the same time points as urine collections were made and serum was separated. Duplicate 0.5-ml aliquots of serum were taken and each added to 4.5 ml water, gelled with 10 ml scintillation fluid, and counted.

Total percutaneous absorption of 17-PT was calculated from the summation of all radioactivity found in the urine and the use of a correction factor to adjust for radioactivity excreted via other routes. The correction factor, i.e., the fraction of a systemically administered dose to be excreted in the urine, was determined in the experiments in which radioactive 17-PT was injected subcutaneously in the monkey as described previously.

Acne Efficacy and Sebum Suppression Studies The efficacy of 17-PT in the treatment of acne was assessed in a placebo-controlled, double-blind study. Forty-four male subjects, ages 14–20, having mild to moderate facial acne and a minimum lesion count of 10 papules/pustules, were recruited and randomly assigned to either active or placebo treatment. For each subject a history was taken and physical examination and screening laboratory evaluations were done prior to the start of the study. No form of acne therapy other than the test medication was allowed during the study, and subjects had to be off all systemic antibiotics for 1 month and all topical medications for 2 weeks prior to initiation of the test therapy.

The study ran for 12 weeks with the test medication being applied three times daily. Subjects were instructed to make the morning and evening applications following a face wash. Efficacy was evaluated by lesion count, and the subjects were seen at 0, 2, 4, 6, 8, and 12 weeks. At each visit all inflammatory lesions on the entire face were counted and recorded. In addition, the oiliness of four sites (chin, cheeks, nose, and forehead) were graded individually by inspection using a 0–3 scale, where 0 represented no oil and 3 represented extreme oiliness. For purposes of statistical analysis the grades for the four sites were summed. Signs of irritation were also noted. At the end of the study the code was broken and statistical evaluation of the results made.

The ability of 17-PT to reduce the rate of sebum excretion was evaluated in a separate double-blind, placebo-controlled study in 20 healthy males, 22–31 years of age, having mild to moderate acne. Each was given a physical examination, including screening laboratory evaluation to insure that they were in good health, then randomly assigned to either active or placebo treatment. All topical therapy must have been discontinued at least 1 week prior to entry into the study.

The study was divided into two phases: a 3-week control period during which only placebo pads were used by all subjects, and a 9-week treatment period during which those assigned to active therapy received drug-containing pads. Application was three times daily for all subjects during both phases. During the 3-week control period, weekly evaluation of the sebum excretion rate (SER) of forehead skin and plasma testosterone was made for the purpose of

Table I. Percutaneous Absorption of 17-PT*

Animal	Site	Method	Total Absorption	
			% Dose	# Expts
Hamster	Gland	in vitro	1.0 \pm 0.3	6
Hamster	Non-gland	in vitro	2.3 \pm 1.3	6
Monkey	Back	in vitro	1.4 \pm 0.9	3
Man	Abdomen	in vitro	0.4 \pm 0.2	3
Man	Face	in vivo	7.7 \pm 3.3	5

* Total absorption is the mean and standard deviation measured as the percent of the applied dose.

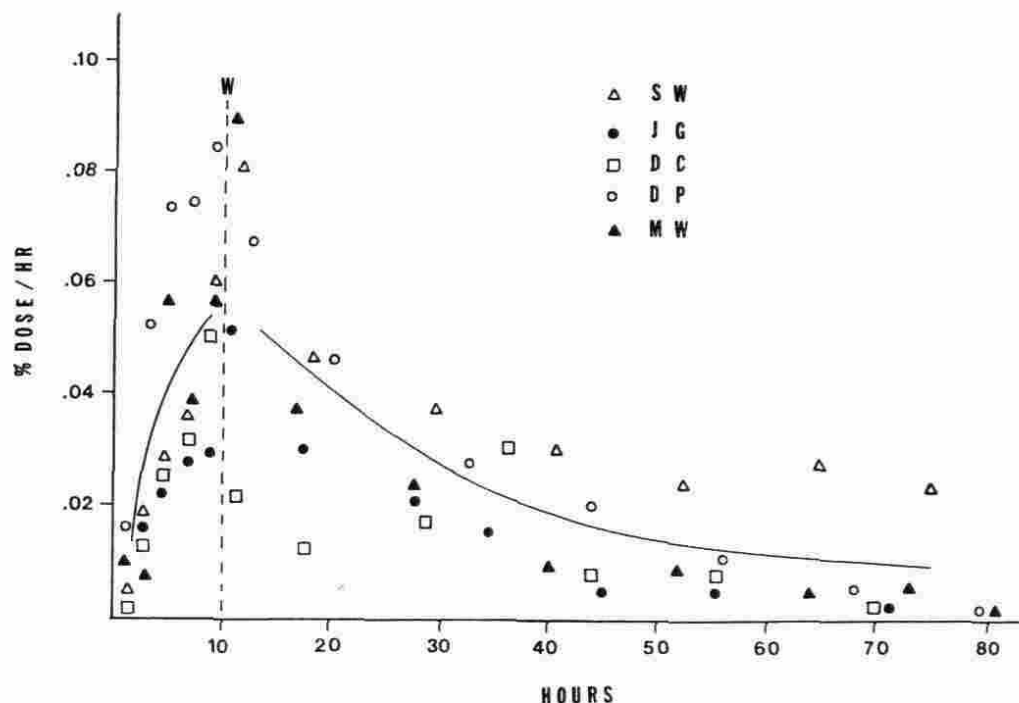


Figure 2. Urinary excretion of ^{14}C -17-PT from its topical application to the face of five human volunteers. The line is the best fit of the individual sample points. W: surface wash time.

establishing baseline values. During the treatment phase these parameters were measured at 4, 6, 8, and 9 weeks.

The SER was determined gravimetrically following a 3-h collection period using a previously published technique [7], and plasma testosterone was determined by radioimmunoassay.

RESULTS

Data obtained on the percutaneous absorption of 17-PT in the hamster, monkey, and man are presented in Table I. 17-PT absorption is generally found to be low, except for human face skin, irrespective of the species examined. Skin overlying the flank organ of the hamster is no more permeable than that of adjacent non-gland skin or that of monkey skin. In fact, skin overlying the flank organ is statistically less permeable ($p=0.05$) than that of adjacent skin (1.0% versus 2.3% of the applied dose). 17-PT absorption through human abdominal skin (0.4%) is even lower than either hamster (1-2.3%) or monkey skin (1.4%). However, 17-PT absorption through face skin is considerably higher (7.7% of the applied dose).

The rate of urinary excretion of radioactivity following application to the face of five volunteers is shown in Fig 2. The rate of excretion rises steadily until the time of the face wash at 10 h, then falls. Serum levels of radioactivity (not shown) follow in a similar

fashion. The highest serum level of 17-PT is seen at 10 h and is equivalent to approximately 10 ng/ml of parent drug (range = 2-22 ng/ml). The amount of radioactivity detected in the serum was too low to attempt to identify the nature of the chemical species carrying the label.

A summary of the results obtained in the acne clinical study is given in Table II. As there were no clinically significant differences seen between the placebo and active formulations at any of the intermediate grading periods, only the data from the baseline and final grading periods are presented.

It can be seen that, with one exception, there are no differences between active and placebo treatment at week 12 in the parameters papules, pustules, or oiliness. With respect to papules and pustules, not even a placebo effect is noted in this study, i.e., there is no improvement from week 0 to 12. A non-significant trend toward improvement in the oiliness score with time is seen for both active and placebo treatment. This effect is probably attributable to the physical removal of surface oil secondary to t.i.d. use of the ethanol saturated pads.

The single parameter in which a statistically significant difference between active and placebo treatment is seen at week 12 is with nodules/cysts (N/C). In view of the fact that the study was not

Table II. Effect of 17-PT on the Treatment of Acne^a

Treatment		Week 0				Week 12			
		Pap ^b	Pust ^c	N/C ^d	Oil	Pap ^b	Pust ^c	N/C ^d	Oil
Placebo	Mean	15.9	5.2	1.0	11.8	19.1	4.7	1.7	6.3
	SD	10.0	3.9	1.9	3.2	8.6	5.5	2.5	6.0
	Mean	20.5	5.6	0.8	9.9	19.3	4.1	0.4	7.1
Active	SD	9.9	4.6	1.8	3.3	12.0	4.6	0.6	5.0

^a Mean and standard deviation of lesion counts and oiliness score.

^b Pap: papules.

^c Pust: pustules.

^d N/C: nodules/cysts.

Table III. Effect of 17-PT on Sebum Excretion Rate*

Treatment		Control Period (week)				Treatment Period (week)			
		1	2	3	Mean	4	6	8	9
Placebo	Mean	2.19	1.95	2.09	2.08	2.36	2.15	2.22	2.22
	SD	0.68	0.55	0.66	0.61	0.81	0.76	0.56	0.62
Active	Mean	2.41	2.44	2.42	2.42	2.16	2.22	2.35	2.34
	SD	0.79	0.83	0.77	0.78	0.74	0.75	0.87	0.79

* Mean and standard deviation of sebum excretion expressed as mg/10 cm²/3 h

designed to primarily examine nodulo-cystic acne and the number of lesions in the N/C category are very small, the difference between treatments is felt to be a chance occurrence only. In keeping with this conclusion it should be noted that the difference between week 0 and week 12 in the N/C count is not statistically significant for either active or placebo therapy.

The effect of 17-PT on the SER is shown in Table III. Nineteen of the twenty test subjects successfully completed the entire course of therapy. One subject was dropped after failing to return for three of the four evaluation visits. No statistically significant differences were observed at any time point between active and placebo therapy, nor were any significant differences observed between the control (mean) and treatment period for either active or placebo therapy. Two subjects in the placebo group experienced some transient facial irritation during the course of the study. However, both were able to continue in the study, and the reactions disappeared without the need for treatment.

The effect of 17-PT on plasma testosterone levels of the same nineteen subjects is presented in Table IV. There were no statistically significant differences between active and placebo therapy at any time point nor were there any significant differences between the control (mean) and treatment period for either active or placebo therapy.

DISCUSSION

The flank organs (costovertebral glands) of the golden Syrian hamster are widely used as a model for the human sebaceous gland [8]. They are grossly visible paired structures, situated on the back of the animal, and contain sebaceous glands, large pigmented hair follicles, and dermal melanocytes. The utility of the flank organ to the dermatopharmacologist derives from the fact that all three of its components are androgen sensitive, although most work to date has focused on the sebaceous glands. In the mature male hamster the sebaceous glands are palpable, 6–8 mm in diameter, and almost fill the entirety of the dermis. In immature males, castrated males, and mature females, the glands are much smaller but can be stimulated to full potential by topical or parenteral administration of testosterone or other androgens.

This response to androgen administration has become the basis of the model and has allowed investigators to systematically screen drugs for their ability to block the stimulatory effect of androgens, i.e., to screen for antiandrogens. The model can be further refined with respect to topically applied antiandrogens in that local-versus-systemic activity can be deduced. Unilateral administration of a systemically active drug will lead to an effect on both glands,

whereas a locally active drug will fail to affect the contralateral gland.

Recent work suggests that the use of the hamster flank organ as a screening model to identify therapeutically effective drugs may be seriously flawed. Vane et al [5] have shown that a retinoid derivative, Ro 15-0778, highly active in the hamster flank organ as well as in castrated testosterone stimulated rats, is without effect in man. More importantly, they have demonstrated that its clinical failure is not a result of poor bioavailability in man. Following 12 weeks of treatment in 10 acne subjects at an oral dose of 11 mg/kg/day, the sebum level of parent drug was found to be 61 ± 49 ng/mg. Although this sebum drug level did not result in a reduced SER, it was equal to the sebum drug level measured in the rat (52 ng/mg), which was associated with a 70%–86% reduction in the rat's SER. Moreover, the sebum drug level seen in man was greater than the 30.5 ng/mg level measured in the hamster flank organ following oral dosing, a level which was associated with a 43% reduction in gland weight. Thus, sebum or tissue levels that are sufficient to produce a pharmacologic effect in the hamster and rat were without effect in man.

Review of the literature disclosed additional examples of model failure. Spironolactone [9,10], cyproterone acetate [11,12,13,14], and flutamide [15,16] all exhibited antiandrogenic activity in the flank organ when applied topically, yet have not shown clinically significant topical efficacy in man. The latter two drugs, however, appear to exert their inhibitory effects on the flank organ via a systemic rather than local route, i.e., bilateral reduction in gland size was noted following unilateral application [9,11,15]. Thus, their clinical failure following topical application may simply reflect the much larger volume of distribution in man.

The data obtained in the present study offer further proof that the hamster flank organ model fails to accurately predict therapeutic efficacy in man. Though active topically in the hamster at doses ranging from 10–250 µg/day (equivalent to solutions ranging from 0.2–5.0% in concentration), in man a 4.0% solution of 17-PT was found to be ineffective in the treatment of acne as well as in the inhibition of SER. Given the known differences in percutaneous absorption between the skin of animals and man, it seemed likely that the therapeutic failure observed in the present study might well be due to low drug permeation in man. However, because direct measurement found human face skin to be more permeable than the skin overlying the flank organ (7.7% versus 1.0% of the dose, respectively), it is clear that differences in absorption cannot account for the observed differences in pharmacologic activity. The possibility of different skin metabolic profiles of 17-PT in hamster and man

Table IV. Effect of 17-PT on Plasma Testosterone*

Treatment		Control Period (week)				Treatment Period (week)			
		1	2	3	Mean	4	6	8	9
Placebo	Mean	0.66	0.64	0.59	0.63	0.58	0.71	0.72	0.69
	SD	0.21	0.18	0.15	0.16	0.11	0.16	0.14	0.18
Active	Mean	0.74	0.68	0.72	0.71	0.61	0.75	0.75	0.73
	SD	0.19	0.16	0.16	0.12	0.10	0.10	0.14	0.09

* Mean and standard deviation expressed as µg/ml serum.

as an explanation of the observed species differences cannot be ruled out at this time.

Although the single failure of the hamster flank organ model reported here does not justify condemnation of the model, when coupled with the recently reported experience on Ro 15-0778 one must seriously question the relevance of the model. 17-PT is an antiandrogen whose activity is dependent upon blockade of the dihydrotestosterone receptor. Ro 15-778 is a retinoid whose mechanism of action is unclear, but which is known to be devoid of antiandrogen activity and, therefore, is acting by a mechanism unrelated to that of 17-PT. Given that two drugs of unrelated structure and pharmacologic activity both fail in man, in spite of unequivocally good activity in the flank organ and comparable topical bioavailability, it is clear that the model has failed.

What is particularly puzzling is the failure of the flank organ model to accurately predict the lack of efficacy of Ro 15-0778, because prior work with retinoids had shown good correlation between the model and clinical efficacy. In a study directly comparing isotretinoin with the aromatic retinoid etretinate, both dosed by subcutaneous injection, Gomez [17] found that isotretinoin significantly suppressed the flank organ, whereas etretinate was virtually without effect. These results parallel the clinical observations of Goldstein et al [18], in which etretinate was found to be much less efficacious than isotretinoin in the treatment of nodulo-cystic acne. The reduction in SER and lesion count at the end of therapy (8 weeks) was 21% and 8% for etretinate, versus 86% and 43% for isotretinoin.

One explanation for the disparate results obtained with the aronoid derivative Ro 15-0778 may be that this drug is not a typical retinoid or, perhaps, not a retinoid at all. In most bioassay systems used to screen retinoids, this drug is inactive. Connor et al [19] have shown that it fails to induce epidermal hyperplasia in the hairless mouse following topical application. Loeliger et al [20] have shown that it fails to induce regression of established skin papillomas in mice following intraperitoneal injection. With the exception of the hamster flank organ, the only test system in which Ro 15-0778 shows activity is in reversal of keratinization in the retinoid-deficient hamster trachea model [21]. Thus, the clinical failure of Ro 15-0778 may have little relevance to the use of the flank organ as an efficacy screen for retinoids.

We do not suggest the abandonment of the flank organ model, but rather a better understanding of its biochemical makeup and pharmacologic response relative to the human sebaceous gland.

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